Biochemical Pharmacology, Vol. 46, No. 1, pp. 131–138, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

EFFECTS OF DOMOATE, GLUTAMATE AND GLUCOSE DEPRIVATION ON CALCIUM UPTAKE BY RAT BRAIN TISSUE *IN VITRO*

M. S. NIJJAR*

Department of Anatomy and Physiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada C1A 4P3

(*Received* 30 *January* 1992; *accepted* 2 *March* 1993)

Abstract—The toxic effects of excitatory amino acids (EAAs) on the central nervous system appear to be mediated by calcium. Calcium uptake into rat brain tissue slices was studied in the absence and in the presence of domoate and glutamate. Calcium uptake into brain cytoplasm was enhanced by domoate in a concentration-dependent manner. Glutamate also stimulated calcium uptake. Calcium uptake into brain tissue was enhanced markedly by the removal of glucose from the Krebs–Henseleit–Ringer bicarbonate incubation medium. Stimulation of calcium uptake by glucose deprivation increased with incubation time, suggesting the depletion of energy stores, i.e. ATP, which is necessary for calcium transport in brain tissue. Replacement of NaCl with choline chloride in the incubation medium also enhanced calcium uptake into brain tissue cytosol. The removal of both glucose and NaCl from the medium produced an additive effect on calcium uptake, indicating independent mechanisms of action. NaF stimulated calcium uptake into brain tissue more in the presence of glucose than in its absence. Since NaF is an inhibitor of glucose metabolism, these results indicate that glucose metabolism is somehow linked to calcium transport in brain tissue. Since ATP is required by calcium pumps, which extrude as well as store calcium in nervous tissue cells, depletion of ATP, either in the absence of glucose or when glucose metabolism is blocked by NaF, may be responsible for the accumulation of calcium in the brain tissue cytosol, and for the neurotoxicity induced by domoate and glutamate.

Calcium plays a central role in the regulation of numerous intracellular processes in the central nervous system [1–4]. Some of these effects are achieved in combination with the ubiquitous protein calmodulin, whereas other effects may be a direct result of calcium [1–4]. The intracellular calcium level undergoes oscillations that are produced by alterations in calcium influx through channels and efflux by the calcium pump in plasma membranes. Calcium is also taken up and released by the endoplasmic reticulum [5, 6]. Certain extracellular agonists stimulate phospholipase C in membranes to produce diacylglycerol and inositol-1,4,5-triphosphate, which mobilizes calcium from the intracellular stores, i.e. the endoplasmic reticulum [7–9]. Any derangement in one or more of these regulatory mechanisms will produce a profound effect on calcium homeostasis and serious metabolic and functional consequences.

Excitatory amino acids (EAAs†) produce toxic effects in the central nervous system [10–12]. The mechanism by which EAAs produce their toxicity in the central nervous system is not clearly defined. However, the available evidence indicates that calcium mediates the toxic effects of EAAs in brain tissue [2, 3, 6]. Depolarization of presynaptic elements causes the influx of calcium [5], which initiates numerous cellular processes including the release of neurotransmitters at the synapses [13, 14] and neuromuscular junctions [15]. The enhanced release of neurotransmitters at the synapse potentiates neurotransmission between adjacent neuronal cells [4] and muscular contraction at the neuromuscular junction [15]. Postsynaptically, an interaction of glutamate with *N*-methyl-D-aspartate (NMDA) receptors enhances the influx of calcium through voltage-dependent calcium channels. Glutamate also interacts with non-NMDA (kainate/quisqualate) receptors that allow rapid influx of sodium causing depolarization and dissociation of magnesium ions from NMDA receptors, thereby activating latent NMDA receptors and further potentiating the influx of calcium and neurotransmission [16]. A high level of calcium [6] and accumulation of glutamate in the synapse produce lesions in brain tissue, resulting in central nervous system dysfunction [10].

In this paper, uptake of radioactive calcium into brain tissue cytoplasmic and membranous compartments was investigated, and the effects of domoate and glutamate were ascertained. The results show that calcium uptake by the brain tissue was influenced profoundly by glucose and NaCl in the absence or presence of these agonists.

EXPERIMENTAL PROCEDURES

Materials. All biochemical reagents used in this study were of analytical grade and were purchased

* Correspondence: Dr. M. S. Nijjar, Department of Anatomy and Physiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, Prince Edward Island, Canada C1A 4P3. Tel. (902) 566-0802; FAX (902) 566-0958.

† Abbreviations: EEAs, excitatory amino acids; NMDA, *N*-methyl-D-aspartate: and KHRB, Krebs–Henseleit–Ringer bicarbonate.

from the Sigma Chemical Co. (St. Louis, MO). Radiolabelled calcium ($^{45}CaCl_2$) was purchased from Dupont Canada Inc. (Mississauga, Ontario). Diaflo ultrafiltration membranes (0.45 μm) were purchased from the Amicon Division of W. R. Grace & Co. (Danvers, MA).

Sprague–Dawley male rats were purchased from Charles River Laboratories (Montreal, Quebec) and kept in animal quarters of the Atlantic Veterinary College with a light and dark cycle of 12 hr each.

Calcium uptake in brain tissue slices. Sprague–Dawley male rats of 200–250 g body weight (8- to 10-weeks-old) were stunned and decapitated, and brain tissue was excised and placed in a beaker containing ice-cold Krebs–Henseleit–Ringer bicarbonate (KHRB) medium (pH 7.4). This medium was prepared by mixing 100 mL of 154 mM NaCl, 4 mL of 154 mM KCl, 3 mL of 110 mM $CaCl_2$, 1 mL of 155 mM KH_2PO_4, 1 mL of 154 mM $MgSO_4 \cdot 7H_2O$, and 21 mL of 154 mM $NaHCO_3$ in a total volume of 130 mL. The mixture was gassed with 5% carbon dioxide to pH 7.4 [17]. The tissue was placed on Whatman filter paper to remove as much fluid as possible, and slices of uniform thickness (1 mm^3) were prepared and placed in a petri dish immersed in chipped ice. Weighed slices (75–100 mg) were placed in labelled glass test tubes in KHRB medium (pH 7.4) containing 10 mM glucose, and flushed with a gas mixture of 95% oxygen and 5% carbon dioxide. The tissue was pre-equilibrated for 3 min at 37°, and radioactive $CaCl_2$ (1.67 Ci/mol) was added to start the reaction. The incubation was carried out in a shaking water bath at 37° under the gas phase of 95% oxygen and 5% carbon dioxide, and terminated by the addition of 2 mL of ice-cold saline, followed by centrifugation for 2 min in a clinical centrifuge, and withdrawal of the supernate. The radiolabelled slices were washed five times with ice-cold saline to remove non-specifically bound radioactivity. The controls contained all the ingredients as the experimentals except that they lacked agonists, and the incubation of tissue slices with radioactive calcium was terminated by the addition of ice-cold saline immediately after the addition of radioactive calcium. The tissue slices were subjected to subcellular fractionation, filtration through a Millipore filter, followed by multiple rinses of membranes on filters with ice-cold saline as described below.

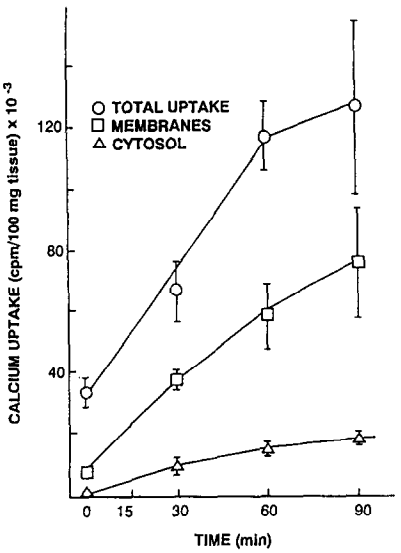

Fig. 1. Calcium uptake into rat brain tissue (○) and cytoplasmic (△) and membranous (□) fractions as a function of incubation time. Approximately 75–100 mg of tissue slices was incubated in KHRB medium, and subcellular fractions were prepared as described in the Experimental Procedures. Some radioactivity in the tissue slices and cytoplasmic and membranous fractions was noted at zero time where the incubation of brain tissue with radioactive calcium was terminated immediately following the addition of radioactive calcium (controls). Results are means ± SD from two experiments performed in triplicate.

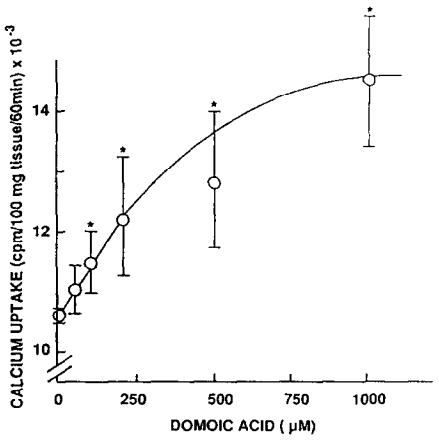

Fig. 2. Calcium uptake into brain tissue cytoplasm as a function of domoate concentration. Incubation conditions and other procedures were identical to those described in the Experimental Procedures and in the legend of Fig. 1, except that the domoate concentration was varied. Results are means ± SD from four experiments run in triplicate for each data point. Data points marked with an asterisk were significantly different from control incubations lacking domoic acid ($P < 0.05$).

Subcellular fractionation and radioactivity measurements. The radiolabelled tissue slices were homogenized in 0.32 M sucrose containing 10 mM Tris–HCl (pH 7.4), and the homogenate was centrifuged at 105,000*g* for 60 min. The supernatant was withdrawn, an aliquot was mixed with the scintillation fluid, and radioactivity was measured in a Packard Tri-Carb 2000CA liquid scintillation analyzer. The pellet was resuspended in the homogenizing medium and filtered through a Millipore filter paper (0.45 μm) under vacuum; then the filter was dried and counted for radioactivity in 5 mL of scintillation fluid. Controls were employed with each set of experimental samples and processed identically; radioactivity in the control samples was subtracted from radioactivity in the experimental samples.

Statistical analysis. Results obtained in this study were analyzed by Student's *t*-test to estimate

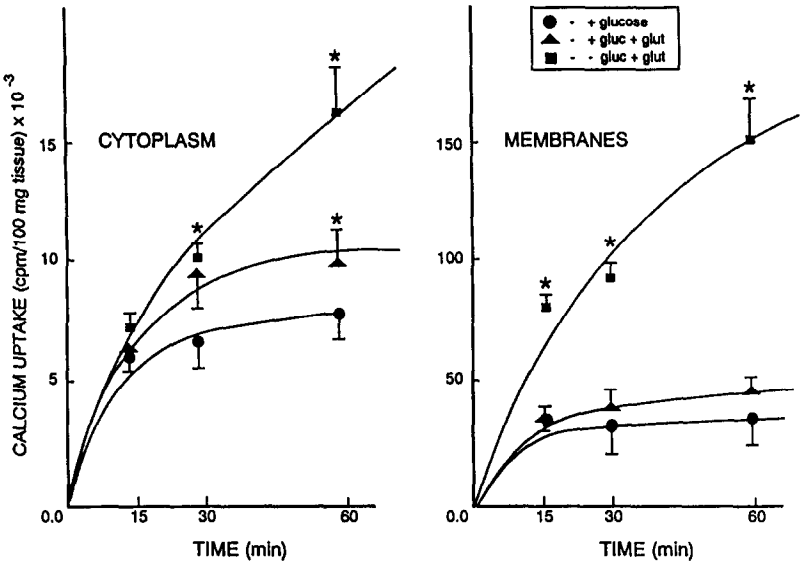

Fig. 3. Calcium uptake into cytoplasmic and membranous fractions from brain tissue in the absence of glutamate (controls) or in the presence of 1 mM glutamate, both in the presence of 10 mM glucose. Other procedures were described in the Experimental Procedures. Results are means ± SD from three experiments run in triplicate. Data points marked with an asterisk were significantly different from the control ($P < 0.05$). Key: (●) plus 10 mM glucose; (▲) plus 10 mM glucose and 1 mM glutamate; and (■) no glucose plus 1 mM glutamate.

statistical significance between the means of different groups [18].

RESULTS

Calcium uptake into brain tissue and into cytoplasmic and membranous fractions increased as a function of time (Fig. 1). Calcium uptake into cytosol of brain tissue was enhanced by domoate (Fig. 2), whereas calcium uptake into membranous fraction was not altered significantly (data not shown). Glutamate (1 mM) also enhanced calcium uptake into cytosol of brain tissue, but its effect on calcium uptake in membranes was marginal (Fig. 3).

When glucose was omitted from the incubation medium, calcium uptake into brain tissue was enhanced markedly (Fig. 3). Calcium uptake into the membranous compartment diminished as the glucose concentration in the medium was increased (Fig. 4, bottom panel), whereas calcium uptake into brain tissue cytosol was not affected significantly (Fig. 4, upper panel). However, when calcium uptake into cytoplasmic fraction was expressed on the basis of per mg protein, it was reduced significantly at 60 min in the presence of 11.1 mM glucose (14.0 ± 6.0 vs $6.7 \pm 1.3 \times 10^3$; $P < 0.05$). Some variation between the replicate samples and different experiments was noted, but this variation was reduced when calcium uptake was calculated on the basis of mg protein.

Calcium uptake into brain tissue cytosol increased as NaCl was replaced gradually with choline chloride in KHRB medium containing 10 mM glucose (Fig. 5, left panel), whereas calcium uptake into membranous fraction was not affected (Fig. 5, right panel).

The effects of glucose deprivation and replacement of NaCl with choline chloride on calcium uptake into cytoplasmic fraction were additive (Figs. 5 and 6, left panels), indicating independent mechanisms of action. The present findings that glucose deprivation affected calcium uptake into membranous fraction most profoundly, whereas replacement of NaCl with choline chloride stimulated calcium uptake primarily in the cytoplasmic fraction (Fig. 5, left panel), further support the view that glucose and NaCl affect calcium uptake into brain tissue by independent mechanisms at different subcellular sites. The effect of NaCl on calcium uptake into brain tissue was not blocked by either ouabain (1 mM) or tetrodotoxin (1 μ) (Table 1). The absence of magnesium or potassium ions from the medium had no significant effect on calcium uptake into brain tissue cytosolic and membranous compartments (data not reported).

The stimulation of calcium uptake into cytoplasmic fraction of brain tissue by the lack of glucose increased with incubation time (Fig. 7, left panel). The stimulation of calcium uptake into membranous fractions peaked at 15- to 30-min of incubation, earlier than the peak in calcium uptake into cytoplasmic fraction at 60 min (Fig. 7, left and right panels). These results suggest that calcium uptake into brain tissue may be linked to the cell energy level, i.e. ATP. NaF in the presence of glucose stimulated calcium uptake into cytoplasmic (8.5 ± 0.4 vs $17.1 \pm 2.2 \times 10^3$; $P < 0.05$) and membranous fractions (6.2 ± 0.7 vs $26.2 \pm 3.0 \times 10^4$; $P < 0.001$)

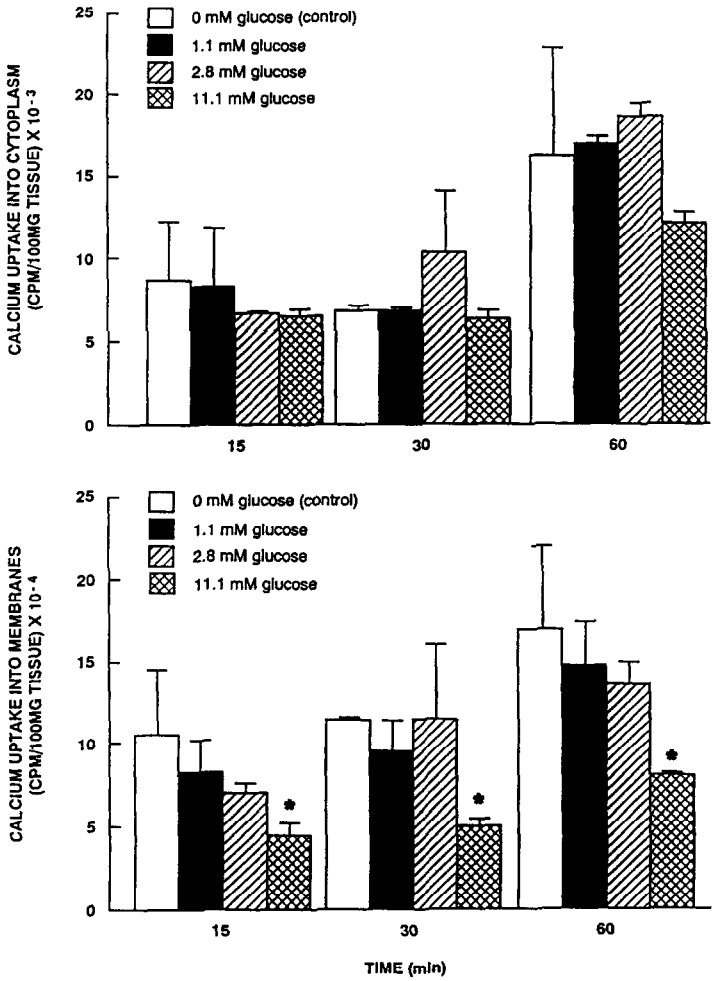

Fig. 4. Effect of variable concentrations of glucose in the incubation medium on calcium uptake into cytoplasmic and membranous fractions from rat brain tissue at different time intervals. Other experimental conditions are described in the Experimental Procedures. Results were obtained from three experiments run in triplicate and expressed as means ± SD. Calcium uptake into membranous fractions at 11.1 mM glucose was significantly different from that in the absence of glucose (0 mM, control) ($P < 0.05$).

(solid bars, Fig. 8), thereby mimicking the stimulation of calcium uptake by the lack of glucose into cytoplasmic (8.5 ± 0.4 vs $12.8 \pm 1.7 \times 10^3$; $P < 0.05$) and membranous fractions (6.2 ± 0.7 vs $14.5 \pm 0.5 \times 10^4$; $P < 0.01$) (left column, Fig. 8). In fact, the stimulation of calcium uptake by NaF in the presence of glucose (solid bars) was higher than the stimulation of calcium uptake by NaF in the absence of glucose (open bars) into cytoplasmic and membranous fractions (Fig. 8). In addition, stimulation of calcium uptake into membranous fraction by NaF in the presence of glucose (320%) was greater than stimulation of calcium uptake by glucose deprivation (152%) (Fig. 8, bottom panel), suggesting an additional site(s) of NaF effect on calcium uptake besides inhibition of glucose metabolism.

Calcium uptake into brain tissue increased as a function of time when the tissue was incubated in the medium containing glucose (control) (Fig. 3). Addition of glutamate stimulated calcium uptake into cytoplasmic fraction at 60 min of incubation. However, removal of glucose from the incubation medium containing a comparable concentration of glutamate markedly stimulated calcium uptake into both the cytoplasmic and membranous compartments of brain tissue (Fig. 3). Calcium uptake into brain cytoplasmic and membranous compartments in response to 250 μM domoate was not altered by glucose (Fig. 9).

DISCUSSION

The results of the present study have revealed that calcium uptake in brain tissue was affected by a variety of agents including some of the excitatory amino acids such as domoate and glutamate. Domoate enhanced calcium uptake into brain tissue

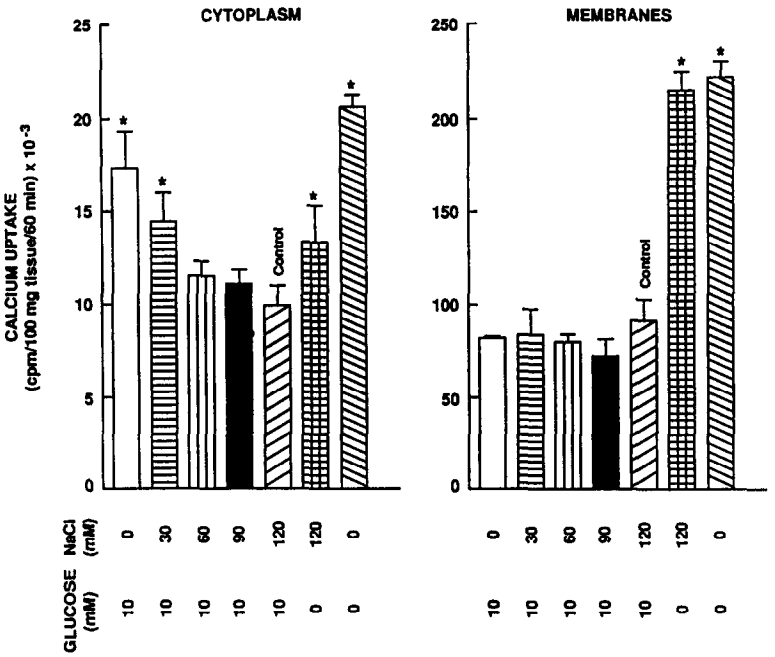

Fig. 5. Calcium uptake into cytoplasmic and membranous fractions of brain tissue as influenced by glucose and NaCl. The incubation containing 10 mM glucose and 120 mM NaCl was considered as the control (▨). NaCl was replaced in the incubation medium with choline chloride, and as NaCl concentration was decreased in the medium, choline chloride concentration was increased. Other experimental conditions were similar to those described in the Experimental Procedures. Results are means ± SD from three independent experiments run in triplicate. Data points marked with an asterisk were significantly different from the controls ($P < 0.05$).

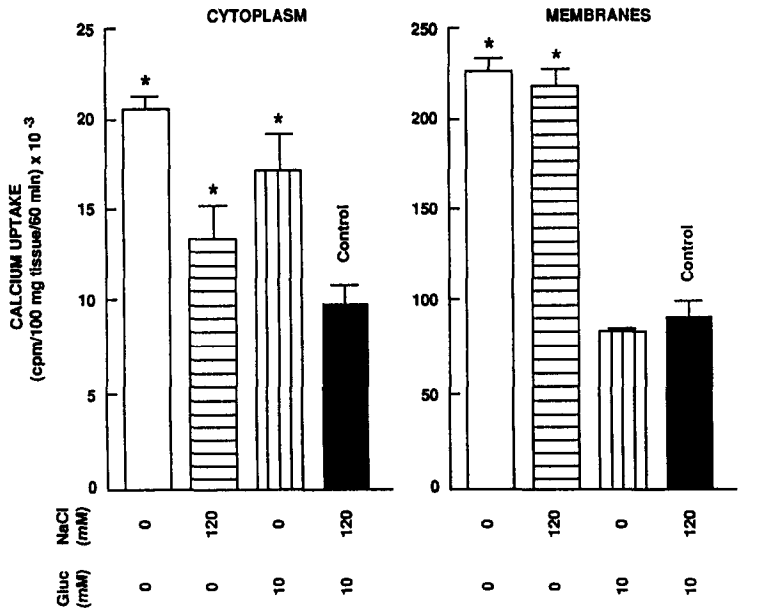

Fig. 6. Calcium uptake into brain cytoplasmic and membranous fractions after incubation for 60 min in KHRB medium in the absence or presence of glucose or NaCl as indicated in the figure. The control incubations contained 10 mM glucose and 120mM NaCl (■). Data are means ± SD from three experiments run in triplicate for each test. Data points marked with an asterisk were significantly different from the control ($P < 0.05$).

Table 1. Effects of ouabain and tetrodotoxin (TTX) on calcium uptake into rat brain tissue slices

Additions	Calcium uptake (cpm/mg tissue/60 min)			
	Cytosol	(% Control)	Membranes	(% Control)
Glucose (10 mM) + NaCl (120 mM)	80 ± 1	(100)	600 ± 40	(100)
Glucose − NaCl	120 ± 7*	(148)	550 ± 60	(93)
Glucose + NaCl + ouabain (1 mM)	80 ± 10	(100)	700 ± 60	(116)
Glucose + NaCl	70 ± 5	(100)	750 ± 70	(100)
Glucose + NaCl + TTX (1 μM)	80 ± 10	(107)	800 ± 100	(107)

* Significantly different from the control ($P < 0.05$). Values are means ± SD of two experiments performed in triplicate.

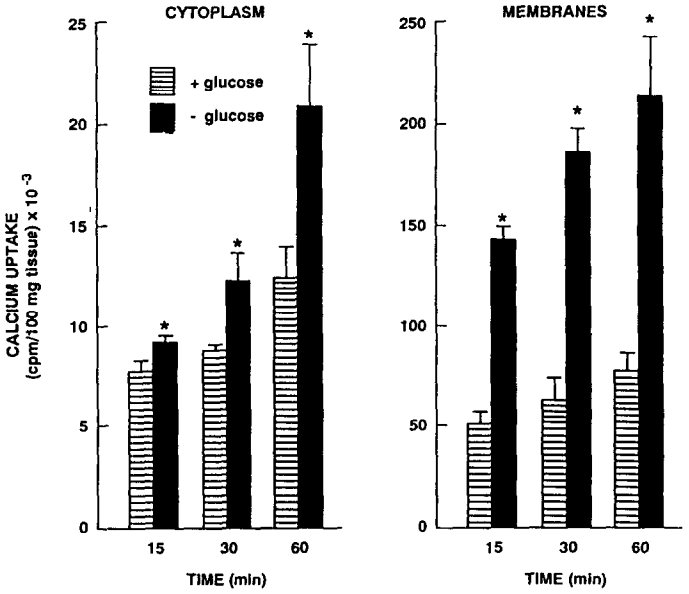

Fig. 7. Effect of glucose deprivation on calcium uptake into brain cytoplasmic and membranous fractions as a function of incubation time. Calcium uptake into brain tissue in the absence of glucose was significantly different from their respective controls ($P < 0.05$). Incubation conditions were identical to those described in the Experimental Procedures. Results are means ± SD from two independent experiments run in triplicate.

cytoplasm, with little or no effect on calcium uptake into the membranous compartment. Glutamate also stimulated calcium uptake into brain tissue cytoplasm, whereas stimulation of calcium uptake into membranous fraction was marginal. These observations are consistent with those reported by other investigators, in that EAAs produce slight to moderate stimulation of calcium uptake in adult brain tissue [5].

Calcium uptake into brain tissue was influenced profoundly by glucose in the KHRB medium used for incubation of brain tissue slices. When glucose was omitted from the medium, calcium uptake into brain tissue was enhanced markedly. The stimulation of calcium uptake in the absence of glucose increased with incubation time. Since the brain tissue relies entirely on carbohydrates as a source of energy [19], these results imply that during the lack of glucose brain tissue cells may have been depleted of ATP, which is necessary for the activity of calcium pumps that extrude calcium from the cells or take up calcium in the endoplasmic reticulum of nervous tissue [6]. A reduction in cellular energy, i.e. ATP, during hypoxia or ischemia has been shown to induce severe deficits in the function of the central nervous system [20, 21]. This concept is further supported by recent findings that glutamate toxicity is enhanced markedly when cerebellar slices are exposed to an oxygen- or glucose-free medium [22].

NaF stimulated calcium uptake more in the

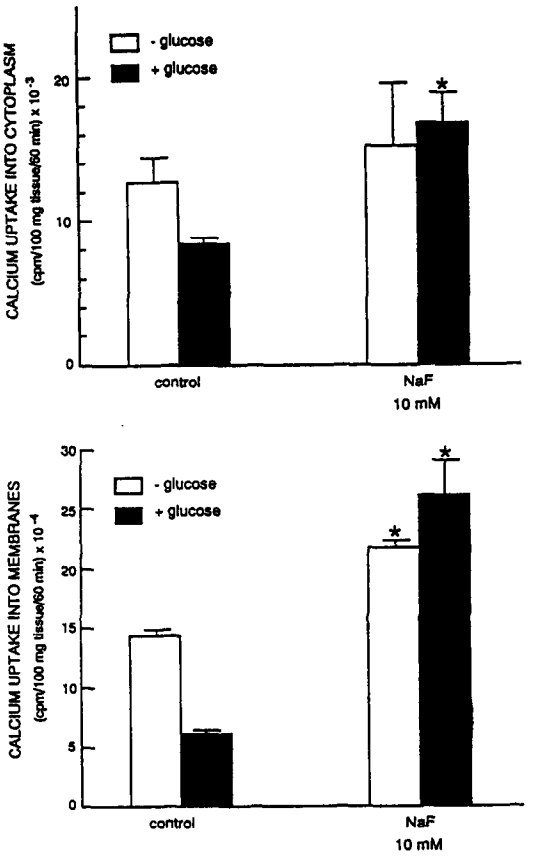

Fig. 8. Effect of NaF in the presence or absence of 10 mM glucose on calcium uptake into cytoplasmic and membranous fractions. Calcium uptake into cytoplasmic and membranous fractions in the presence or absence of 10 mM glucose without NaF (control) is also given. All other experimental conditions are described in the Experimental Procedures. Each data point (mean ± SD) represents calcium uptake in three different experiments measured in triplicate. Bars marked with an asterisk indicate data significantly different from the controls ($P < 0.05$).

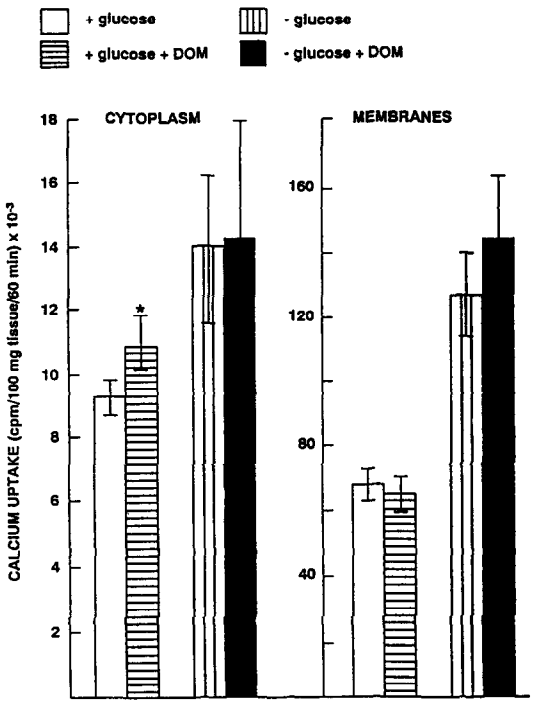

Fig. 9. Effect of 250 μM domoate on calcium uptake into brain cytoplasmic and membranous fractions in the absence (▥) or presence (□) of 10 mM glucose. Results are means ± SD from two experiments run in triplicate. The data point marked with an asterisk was significantly different from the control incubation containing 10 mM glucose ($P < 0.05$).

presence of glucose than in its absence. Since NaF is a potent inhibitor of glucose metabolism through the glycolytic pathways [23], these findings further strengthen the view that glucose deprivation most likely resulted in depletion of the high energy intermediates such as ATP and phosphocreatine. As a result, activity of calcium pumps was diminished, and extrusion of calcium from the cell as well as its uptake into the endoplasmic reticulum was reduced, resulting in an elevation of intracellular calcium. At high levels, calcium is known to be toxic in different tissues including the brain [24]. The present findings that NaF stimulated calcium uptake much more in the presence of glucose than in its absence indicate that NaF stimulated calcium uptake into brain tissue by inhibiting glucose metabolism, but also by acting at another site(s) independent of glucose metabolism.

Calcium uptake into brain tissue was also enhanced when NaCl was replaced by choline chloride in the incubation medium, and decreased when NaCl was reintroduced in a concentration-dependent manner (Fig. 5). This effect of NaCl was not prevented by either ouabain or tetrodotoxin (Table 1), indicating that the effects of NaCl on calcium uptake in brain tissue may not be mediated by either the sodium pump or sodium channels in the nervous tissue cell membranes.

NaCl may have produced synergistic effects on calcium uptake with the transport of glucose or calcium in brain tissue. The effects of glucose and NaCl on calcium uptake into brain tissue were additive, suggesting independent mechanisms of action. Glucose deprivation markedly stimulated calcium uptake into membranous fraction with little effect on calcium uptake into brain cytoplasm. Removal of NaCl, on the other hand, stimulated calcium uptake into the cytoplasmic fraction without any effect on calcium uptake by membranes. These results provide further support for independent sites of action of glucose and NaCl on calcium uptake into brain tissue. They also suggest a specific intracellular site of action of NaCl on calcium uptake. The entry of sodium ions into nervous tissue cells may reduce the electronegative potential gradient across membranes, which provides, in part, the driving force for calcium to enter the cell [6]. In contrast, the effect of glucose on calcium uptake appears to be general in that it affected calcium

uptake into membranous fraction with slight increase in calcium uptake into cytoplasmic compartments. This effect was most likely mediated by the diminished level of ATP.

Calcium uptake into brain tissue was stimulated more by NaF in the presence of glucose than by glucose deprivation from the medium. Apparently, the stimulation of calcium uptake by NaF cannot be fully explained on the basis of inhibition of glucose metabolism. NaF is a potent stimulant of adenylate cyclase activity in brain tissue [25], which utilizes ATP to form cyclic AMP. A rise in cyclic AMP promotes phosphorylation of voltage-dependent calcium channels, further utilizing ATP and promoting the influx of calcium [26, 27].

It is well established that an adequate energy is critically important for the cell ionic homeostasis [see reviews in Refs. 2 and 6]. A drop in cell energy, i.e. ATP, due to lack of glucose impairs the function of calcium and sodium-potassium pumps, thereby altering the fluxes of different ions across plasma membranes. These alterations in ionic homeostasis as a result of diminution of ATP are considered to be primarily responsible for the lesions in brain tissue produced during hypoglycemia, anoxia, hypoxia/ischemia and epilepsy [6, 8–12, 20–22]. The enhanced influx of calcium into brain tissue resulting from the lack of glucose may further aggravate the insult to brain tissue.

Acknowledgements—This work was supported in parts by the Atlantic Canada Opportunity Agency in partnership with the Atlantic Mussel Growers Association, and the National Research Council of Canada (IRAP-H program). The excellent technical assistance of Bruce Wonnacot and Alfred Mitchell in this study is greatly appreciated.

REFERENCES

1. McGeer PL, Eccles JC and McGeer EG, Calcium and its binding proteins. *Molecular Neurobiology of the Mammalian Brain*, pp. 168–170. Plenum Press, New York, 1987.
2. Meyer FB, Calcium, neuronal hyperexcitability and ischemic injury. *Brain Res Rev* **14**: 227–243, 1989.
3. Kennedy MB, Regulation of neuronal function by calcium. *Trends Neurosci* **12**: 417–420, 1989.
4. Miller RJ, Calcium signalling in neurons. *Trends Neurosci* **11**: 415–419, 1988.
5. Benavides J, Claustre Y and Scatton B, L-Glutamate increases internal free calcium levels in synaptoneurosomes from immature rat brain via quisqualate receptors. *J Neurochem* **8**: 3607–3615, 1988.
6. Siesjo BK, Bengtsson F, Grampp W and Theander S, Calcium, excitotoxins, and neuronal death in the brain. *Ann NY Acad Sci* **568**: 234–251, 1990.
7. Sladeczek F, Pin JP, Recasens M, Bockaert J and Weiss S, Glutamate stimulates inositol phosphate formation in striatal neurons. *Nature* **317**: 717–719, 1985.
8. Nicoletti F, Iadarola MJ, Wroblewski JT and Costa E, Excitatory amino acid recognition sites coupled with inositol phosphate metabolism: Developmental changes and interaction with α_1-adrenoceptors. *Proc Natl Acad Sci USA* **83**: 1931–1935, 1986.
9. Baudry M, Evans J and Lynch G, Excitatory amino acids inhibit stimulation of phosphatidylinositol metabolism by aminergic agonists in hippocampus. *Nature* **319**: 329–331, 1986.
10. Olney JW, Glutamate, a neurotoxic transmitter. *J Child Neurol* **4**: 218–226, 1989.
11. Olney JW, Neurotoxicity of excitatory amino acid neurotransmitters. In: *Kainic Acid as a Tool in Neurobiology* (Eds. McGeer EG, Olney JW and McGeer PL), pp. 95–122. Raven Press, New York, 1978.
12. Garthwaite J, Garthwaite G and Hajos F, Amino acid neurotoxicity: Relationship to neuronal depolarization in rat cerebellar slices. *Neuroscience* **18**: 449–460, 1986.
13. Steinhardt RA and Alderton JM, Calmodulin confers calcium sensitivity on secretory exocytosis. *Nature* **295**: 154–155, 1982.
14. Delorenzo RJ, The calmodulin hypothesis of neurotransmission. *Cell Calcium* **2**: 365–385, 1981.
15. Maeda M, Koduma T, Saito M, Tanaka T, Yoshizumi H, Nomoto K and Fujita T, Neuromuscular action of insecticidal domoic acid on the American cockroach. *Pestic Biochem Physiol* **28**: 85–92, 1987.
16. Novelli A, Kispert J, Reilly A and Zitko V, Excitatory amino acid toxicity in cerebellar granule cells in primary culture. *Can Dis Wkly Rep* **16** (Suppl 1E): 83–89, 1990.
17. Dawson RMC, Elliott DC, Elliott WH and Jones KM, Physiological media. *Data for Biochemical Research*, 3rd Edn, p. 446. Clarendon Press, Oxford, 1986.
18. Motulsky HJ, Data analysis. In: *GRAPHPAD Software* (Ed. DiMaggio M), pp. 6–17. Institute of Scientific Information, Philadelphia, 1987.
19. White A, Handler P and Smith EL, Metabolism of brain. *Principles of Biochemistry*, 3rd Edn, pp. 758–763. McGraw Hill, New York, 1964.
20. Luhmann HJ and Heinemann U, Hypoxia-induced functional alterations in adult rat neocortex. *J Neurophysiol* **67**: 798–811, 1992.
21. Hashimoto K, Kikuchi H, Ishikawa M and Kobayashi S, Changes in cerebral energy metabolism and calcium levels in relation to delayed neuronal death after ischemia. *Neurosci Lett* **137**: 165–168, 1992.
22. Garthwaite G, Williams GD and Garthwaite J, Glutamate toxicity: An experimental and theoretical analysis. *Eur J Neurosci* **4**: 353–360, 1992.
23. White A, Handler P and Smith EL, *Principles of Biochemistry*, 3rd Edn, p. 374. McGraw Hill, New York, 1964.
24. Verity MA, Ca^{2+}-dependent processes as mediators of neurotoxicity. *Neurotoxicology* **13**: 139–148, 1992.
25. Nijjar MS, Nature of the cytoplasmic factor(s) modulating adenylate cyclase in the developing rat brain. *J Neurochem* **34**: 1304–1308, 1980.
26. Hescheler J and Rosenthal W, G-Protein control of voltage-dependent Ca^{2+} currents. In: *G Proteins and Calcium Signalling* (Ed. Naccache PH), pp. 79–94. CRC Press, Boca Raton, FL, 1990.
27. Gray R and Johnston D, Noradrenaline and β-adrenoceptor agonists increase activity of voltage-dependent calcium channels in hippocampal neurons. *Nature* **327**: 620–622, 1987.